REMARKS

Status of Claims

Claims 1-15 are pending in this application. Claim 1 has been amended to more particularly point out that which applicants regard as their invention. Support for the amendment can be found, *inter alia*, in paragraph [0042] of the specification.

Claim Rejections under 35 U.S.C. § 102

The rejection of claims 1-5, 7, 9, 12 and 14 under 35 U.S.C. §102(b) over Hubner et al. (Biochemistry, 1999, vol. 38, pp 1371-1376) is respectfully traversed with respect to the amended claims and in view of the following remarks.

The present invention is drawn to a method for identifying biomolecules in variant libraries. This method involves producing a variant library consisting of a number of variants of gene sequences coding for the biomolecule, and dividing that library into compartments of a microtiter plate. Once divided, the next step is producing the biomolecules and testing the phenotypes to look for a specific type. Once the specific phenotype is found, the selected compartment is further divided into different compartments, with this step being repeated until every compartment contains only one variant.

Hubner et al. is directed to modifications of ribonuclease T1 specificity, and discloses an assay for the screening of a single clone to isolate a desired phenotype (see abstract; p. 1372, right column). In order to screen for the desired phenotype, in particular RNA hydrolysis activity, the transformed cells are incubated on agar indicator plates.

The method according to the present invention differs from the method of Hubner et al. in at least steps b), c), and f).

In step b) of the present invention, the variant library is divided into a number of compartments of a microtiter plate and a deep well plate, respectively.

The variants are divided into compartments of a microtiter plate that, unlike Hubner et al., intentionally comprise multiple variants, B_0/W_0. Hubner et al., in contrast, is completely silent on the use of a microtiter plate or deep well plate for the division of the variant library. The Office Action defines the "compartment" in Hubner et al. as the agar plate on which the library is divided, which allows for isolation of a single phenotype in one step because of the ability to isolate a single clone from a single colony on the plate.

Additionally, even if the agar plates used to divide the library in Hubner et al. were considered to be compartments for the division of the library, the reference is also silent about the ratio of the number of compartments and the number of variants in the library, and further, on the number of compartments itself. This is in stark contrast to the clearly defined ratios in claim 1 of the present invention.

In step c) of the present invention, the produced biomolecules are tested and screened for a particular phenotype, whereas the genotype cannot be determined solely on the observed phenotype. This is in direct contrast to the method disclosed in Hubner et al.

Hubner et al. discloses that RNAse variants are expressed and secreted by *E.coli* cells and the active RNAses could be detected on RNAse indicator agar plates (see 1373, right column). Hubner et al. further discloses that a large number of variants could be tested for RNA hydrolysis by colony screening and 180 active variants were detected in several rounds of transformations and platings from a total number of $1.6x10^6$ independent transformants (see page 1373, right column). The goal of the plating of these variants on agar plates is to separate a single clone from the other variants and to test the separated clone for a specific phenotype. Using this method, all single phenotypes and their related genotypes are separated from the rest of the variants in a single step. It is evident from the method described in Hubner et al. that the total number of independent transformants were plated as single colonies, and therefore, each colony corresponds to a single clone. The subsequent tests in Hubner et al.,

which test the phenotype, allow for direct conclusions to be made on the genotype based solely on the observed phenotype.

Hubner et al. can be distinguished from the present invention in this regard because no direct conclusions on the genotype can be made based on the observed phenotype. In other words, the genotype and phenotype are uncoupled from each other.

In step f) of the present invention, steps c) through e) are repeated to the n-fold until every compartment maximally contains only one variant ($k_n<=1$) of the gene sequence coding for the biomolecule. Due to this n-fold repetition, the method is an iterative method. Therefore, the number of genotypes correlated to a certain phenotype decreases every time steps c) through e) are repeated. At the end of the iterative method, a complete separation of the variants is achieved, and it is only at this point that the coupling of the phenotype and genotype is retrieved.

In contrast to the present invention, Hubner et al. does not disclose an iterative method because it does not contain an n-fold repetition of the single steps of the method. As discussed above, the genotype and phenotype are coupled so that direct conclusions on the genotype can be made based on the observed phenotype at any point during the method. The Office Action points to the "rounds of transformations" as described by Hubner et al, as an indication of an n-fold repetition, however, this statement does not accurately describe the teaching of Hubner et al. Instead, the "rounds of transformations" described in Hubner et al. merely relate to the repeated generation of partial libraries on agar plates due to the long lasting screening procedure and the limitations associated with plating cells on agar indicator plates.

The foregoing demonstrates that Hubner et al. does not teach each and every limitation of Applicants' method and therefore does not anticipate the invention as claimed. Applicants request that the Examiner reconsider and withdraw the rejection of claims 1-5, 7, 9, 12 and 14 under 35 U.S.C. §102(b) in view of Hubner et al.

Claim Rejections under 35 U.S.C. § 103

The rejection of claims 6, 8, 11 and 13 under 35 U.S.C. § 103(a) over Hubner et al. as applied to claims 1 and 7 in combination with Selifonov (WO 01/12791 A1); the rejection of claim 10 under 35 U.S.C. § 103(a) over Hubner et al. in combination with Napolitano (The Journal of Neuroscience, 1987, vol. 7, pp 2590-2599); and the rejection of claim 15 under 35 U.S.C. § 103(a) over Hubner et al. in view of Korn et al. (Methods in Enxymology, 2001, vol. 34, pp 142-153) are each respectfully traversed.

The present invention is directed to a method of identifying biomolecules in variant libraries of biomolecules. This method starts with a large library, which contains the desired variant. After completion of the selection method of the present invention, the variant is in its final desired state and does not need to be further improved through additional cycles of mutation and recombination (see paragraph [0024]). This feature of the present invention is an important advantage in comparison to conventional methods of isolating a single clone, which must contain mutagenesis and selection steps. Because each compartment of the microtiter plate contains more than one clone of the variant library, and the observed phenotype of the compartment is the result of the sum of the clones contained in the compartment, no conclusion can be made from the observed phenotype to the genotype (see paragraph [0051]). Even though the genotype and phenotype of the clones are uncoupled, it is still possible, using the method of the present invention, to retrieve and isolate the clone responsible for the desired property. The fact that it is possible to retrieve the single clone responsible for the desired property from the mixture of clones with a screening method in which the genotype and phenotype are uncoupled, is a surprising result to one of ordinary skill in the art. All previously known methods of

screening and isolation are based on the coupling of the genotype and phenotype (see paragraph [0052]).

Hubner et al. is directed to a method of screening for RNA hydrolysis activity and isolating variants with desired characteristic. In order to achieve this end result, the library is plated on agar indicator plates. Once on the agar indicator plates, the desired colonies are identified based on red haloes forming around the colony. A limitation of this method is the amount of library that can be plated on a single agar plate because of the way in which desired variants are identified.

The success of this method is dependent on a distinct distance being present between the bacterial colonies so that the identifying colored haloes can be seen and distinguished. There are two reasons why the distinct distance between the colonies is imperative to have success with the method taught in Hubner et al. First, it is necessary to have a distance between colonies in order to identify the red haloes around the desired colonies and to distinguish between the different colors present on the plate (i.e. red and blue agar areas). And second, if the bacterial clones are too close together, they stop growing and start lysis. If lysis occurs, intracellular host-own RNAses are released to produce a halo, which appears on the plate as one halo over the whole plate. When this happens, it is not possible to identify single RNAse-secreting clones.

The Office Action theorizes that the colored area of the lawn of transformed cells that comprise a positive red halo that can be picked can be interpreted as a compartment (W_0). And the colored bacteria picked from the compartment are grown in a liquid media with ampicillin, then DNA plasmid is isolated, and then transformed and plated again for selection. The Office Action further asserts that the lawn is not an individual colony on the first plating and thus requires several rounds of transformation and plating to identify one individual unique clone (see page 8, third paragraph of Office Action). Applicants respectfully disagree with these assertions.

Nowhere in Hunber et al. is the process of taking parts of a lawn and transferring them to a new plate to further individualize the clones described. Additionally, Hubner et al. does not disclose the amplification of partial libraries or even dilution of partial libraries. Also, no plasmid preparation of selected parts of the library and re-transformation is described in Hubner et al.

Contrary to the statement in the Office Action that the reference discloses several rounds of transformations, the description of "rounds of transformations and plating on RNAse indicator plates" in Hubner et al. does not mean that the transformation is repeated. Instead, the reference is describing a repetition of the generation of partial libraries on agar plates. This repetition is necessary because the screening work must be divided into smaller procedures because of the maximum capacity of cells that can be plated on the agar plates and still result in individual colonies. The "rounds of transformations" described in the reference is merely referring to the repetition of the screening procedure and not of new transformations. Because the assay in Hubner et al. deals with selecting single clones on a plate, the genotype and phenotype are coupled to each other throughout the entire screening method. This method is clearly different than the method of the present invention which uncouples the genotype and phenotype through the selection process.

The combination of Selifonov et al. does not remedy Hubner et al.'s deficiencies because Selifonov et al. also describes a method of plating transformed cells, and which result in single clones that are assayed for the desired phenotype. Similar to Hubner et al., Selifonov et al. describes plating the library on "solid media to produce *individual* colonies" (emphasis added) (see page 95, line 15). The result of plating the library to isolate single colonies is the coupling of the genotype and phenotype because the single colony can be referred back to a single clone, and because the selected phenotype is associated with the genotype of a particular clone.

In contrast to Hubner et al. and Selifonov et al., the claimed method involves assaying the phenotype for a mixture of variants. The detected

phenotype can not be associated with a particular genotype because the detected phenotype is the product of all the clones of the compartment, i.e. from the sum of all single phenotypes of these clones. The number of clones in the subsequent compartments decreases every time steps c) to e) are repeated until a single clone remains. It is only after steps c) through e) have been repeated enough times such that a compartment has, maximally, only one variant that the detected phenotype is once again coupled to a particular genotype.

Selifonov et al. in combination with Hubner et al. does not teach or suggest a method wherein the phenotype and genotype are uncoupled, nor does their combination provide any teaching or suggestion that the phenotype and genotype should be uncoupled, or how an uncoupling of genotype and phenotype can be achieved. As such, Hubner et al. in combination with Selifonov et al. fail to teach or suggest the invention as claimed and does not render the claimed invention obvious.

The addition of Napolitano et al. likewise fails to remedy the deficiencies of Hubner et al. described above. Nepolitano et al. relate to the complete amino acid sequence and in vitro expression of Rat NF-M. Like Hubner et al., Nepolitano et al. do not teach or suggest a method wherein phenotype and genotype are uncoupled. Nor do they teach or suggest that the phenotype and genotype should be uncoupled or how such uncoupling would be achieved. Thus the combination of Hubner et al. and Nepolitano et al. fails to render the invention as claimed obvious.

Finally, the addition of Korn et al. also fails to remedy the deficiencies of Hubner et al. described above. Korn et al. relates to ribonuclease assays utilizing toluidine blue indicator plates. Like Hubner et al., Korn et al. do not teach or suggest a method wherein the phenotype and genotype are uncoupled. Korn et al. also do not teach that the genotype and phenotype should be uncoupled, or how such uncoupling would be achieved. Thus the combination of Hubner et al. and Korn et al. fail to render the invention as claimed obvious.

Reconsideration and withdrawal of the obviousness rejections are accordingly, respectfully requested.

Conclusion

For the foregoing reasons, the application is respectfully requested to be in condition for allowance, and prompt, favorable action thereon is earnestly solicited.

If there are any questions regarding this response or the application in general, a telephone call to the undersigned at (202) 624-2845 would be appreciated since this should expedite the prosecution of the application for all concerned.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323, Docket No. 102520.57766US.

Respectfully submitted,

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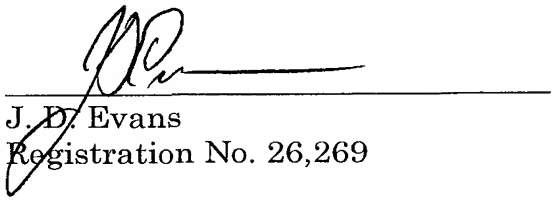

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